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Low Dose Ferrous Gluconate Supplement Fails to Alter the Iron Status of Female Officers-in-Training

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ABSTRACT

Physical training creates an iron cost for the body, which is a risk for young women. This study investigated a low-dose iron supplement for prevention or treatment of iron-deficiency among female RMC staff cadets and ADFA officer cadets and in so doing improve measures of fatigue, general health, physical fitness and increase participation in leisure activities. Cadets consumed either a low dose iron supplement (18 mg iron) or placebo for 13 weeks, using a double-blind, placebo-controlled randomised design. Tests at baseline, 6 wks and 13 wks determined the effects of supplement versus placebo on iron status and other measures. There was no evidence of benefit derived from the iron supplement, although emotional fatigue might have responded positively. The fatigue, health and leisure activity measures remained stable. Physical fitness improved at 6wks, but the improvement had been lost by 13 wks. Early in the semester, when cadets were most physically active, there was a mean decline in iron status as iron was mobilised from liver stores to the tissues. By the end of the semester the apparent loss from iron stores had been replenished. However more than half of the young women commenced the study with iron deficiency to some degree and this situation did not change at the 6 wk or 13 wk testing points. Self-administration of iron supplements is not recommended for the prevention or treatment of iron deficiency. The implementation of nutrition and iron-status monitoring programs are recommended.

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Executive Summary

Iron deficiency is considered the most prevalent nutrient deficiency in the world, with young women particularly at risk. Iron deficiency anaemia impairs performance because low haemoglobin concentration results in reduced supply of oxygen to the tissues. Iron deficiency (without anaemia) is also believed to have a negative effect on physical performance. Iron is also involved in biological activities related to immunity, cognitive functioning and thermoregulation, and iron deficiency has been associated with non-specific symptoms such as fatigue, lethargy, weakness, headaches, irritability and dizziness³. Physical training is believed to create an iron cost for the body. This is a significant risk for young women commencing a physical training program; therefore iron deficiency or depletion may be a preventable cause of the loss of females from the ADF.

Participants were sought among female Royal Military College, Duntroon (RMC) staff cadets and Australian Defence Force Academy (ADFA) officer cadets, with strict criteria set for eligibility to volunteer. Participants consumed either a low dose iron supplement (18 mg iron) or placebo for 13 weeks, using a double-blind, placebo-controlled randomised block design. Participants were tested at baseline, 6 wks and 13 wks to determine the effects of supplement versus placebo on iron status, fatigue, general health, physical fitness and leisure activity.

There was no evidence of differences between the treatment and placebo groups for the biochemical, dietary, physiological or behavioural measures, except for emotional fatigue, which decreased over time for the treatment group. There was evidence of changed concentrations of the iron status measured over the study. Early in the semester, when cadets were most physically active, there was a mean decline in iron status (serum ferritin) as iron was mobilised from liver stores to the tissues. By the end of the semester the apparent loss from iron stores had been replenished.

The prevalence of iron deficiency among the group was higher than other national surveys for this age group. More than half of the young women commenced the study with iron deficiency to some degree and this situation did not change at the 6 wk or 13 wk testing points.

One third of the officer cadets were at risk of eating insufficient dietary iron to meet their nutritional requirement, however total dietary iron was not significantly associated with iron status. There was a large variation in physical activity due to individual university timetables. Fitness (shuttle run) improved at mid-point then declined by the final testing point; this variation did not correlate with changes in iron status, fatigue or leisure activity. Leisure activity and fatigue (mental, vigour, physical) scores were stable. Alcohol consumption correlated negatively with iron status, possibly as a result of disordered eating habits among the heavier drinkers.

Conclusions Low-dose iron supplementation is ineffective at improving iron status, physical performance or psychological status of female officers-in-training. Iron status is

not associated with physical fitness, mental or physical fatigue, as measured in this study. Iron status declined in the first half then recovered in the second half of the semester. More than half of the young women involved in this study had low iron stores and ~6% were anaemic. Those who drank more than 15 g alcohol per day were more likely to be iron deficient.

The following recommendations have been made.

1. We recommend a nutrition education program, which highlights the need for female staff and officer cadets to choose good sources of iron in their daily diet and reduce their level of alcohol consumption.
2. Female officers-in-training should have their iron status assessed.
3. Diagnosed problems of iron-deficiency (and iron overload) should be referred for medical attention. Self-administration of iron supplements, even low dose, is not recommended. Supplemental iron, preferably a controlled-release form providing 30–100 mg of iron per day, should only be administered under medical supervision.

Authors

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Julia Carins, BSc, began work at the Defence Nutrition Research Centre in 1996, and since that time has been involved with many research projects of varying nature. Whilst at DSTO-Scottsdale, she has undertaken work in the Food Technology, Nutrition, Chemistry and Microbiology areas, including projects on fresh feeding, rationing, food acceptability, shelf life extension, vitamin analysis, predictive microbiology.

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1. Introduction

1.1 Iron deficiency

Iron deficiency is still considered the most prevalent nutrient deficiency in the world, affecting 10-20% of the population [1]. Low dietary iron intake is common in many developed nations, particularly amongst women. A survey of Australian adults revealed that 50% of women aged 19 to 24 years had a daily iron intake below the recommended level of 12-16 mg [2]. Young women are particularly at risk, not only because of low dietary intake, but also due to a higher iron requirement created by growth, puberty and the onset of menstruation. Iron deficiency has been reported to affect at least 8% of Australian women [3], with the prevalence of anaemia being approximately 2-5%. Other evidence has shown prevalence of iron deficiency in women in the 15 to 30 year age group as high as 12.5% (as indicated by low serum ferritin) [4].

Iron is required for the synthesis of haemoglobin, a blood protein which is the oxygen transport component of blood. Iron deficiency occurs in three stages. The first stage is characterised by low iron stores, as indicated by low serum ferritin (Frt). In the second stage erythropoiesis (red blood cell production) decreases. The final and most severe stage of iron deficiency, anaemia, involves abnormally low haemoglobin (Hb) in the blood. Iron deficiency anaemia impairs performance because the reduced haemoglobin concentration results in reduced oxygen carrying capacity, so the supply of oxygen to the tissues for the release of energy is reduced. This has a negative effect on physical fitness through reduced aerobic capacity [5]. Iron is also involved in biological activities related to immunity, cognitive functioning and thermoregulation.

There is some evidence that iron deficiency (without anaemia) can also have a negative effect on physical performance, particularly on endurance [6]. For example, the iron status of a group of British Royal Engineers was positively associated with aerobic fitness (multistage fitness test) during adventurous training in the hot wet tropics [7]. Energetic efficiency, productivity and voluntary activity levels are also believed to be reduced when iron deficiency is present. Many other non-specific symptoms such as fatigue, lethargy, weakness, headaches, irritability and dizziness have been associated with iron deficiency. These symptoms all have the potential to affect general health, and reduce the quality of life in terms of work, leisure, social activities and family responsibilities.

1.2 Iron cost of physical training

Physical training is believed to create an iron cost for the body. Athletes have been reported to have a higher prevalence of iron deficiency—as high as 80% in women endurance athletes [8]. Proposed mechanisms for iron deficiency in athletes include haemodilution, foot strike haemolysis, iron losses in urine, faeces or sweat, and malabsorption of iron [9]. Inadequate dietary intake may also contribute, because carbohydrate intakes are increased to meet the energy needs of the athlete and carbohydrate foods are generally not rich sources of iron.

This increased incidence of iron deficiency creates significant risks for young women entering a physical training program. The effects of iron deficiency on physical fitness may reduce their ability to complete training, or to meet selection criteria, or to pass milestones within the training. The non-specific symptoms may reduce their general health, mental

commitment to training, and their belief in their ability to complete training (e.g. military physical fitness training) or to compete in their chosen sport.

The commencement of physical training by ADF female recruits or officers-in-training puts them at risk of developing iron deficiency. If they do become iron deficient they may not be able to complete training, or may fail required fitness tests. Further, they may suffer non-specific symptoms such as fatigue that may interfere with their other work duties, social and family life. All these problems increase the likelihood that female recruits and officers-in-training will lose confidence in their ability to do the job, and that they will be lost from the ADF.

1.3 Treatment of iron deficiency

The first approach to treatment of iron deficiency is the elimination of any source of excess blood loss. Because of a high prevalence of upper gastrointestinal disease, gynaecological problems and colorectal disorders found in premenopausal women with iron deficiency anaemia, it is recommended that upper endoscopic examination be considered in the examination of this group, particularly where there are digestive complaints or iron deficiency that is refractory to iron supplementation [10].

However, it should be stressed that because haemochromatosis has a prevalence of 1 in 300 in the Australian Caucasian community, treatment with iron supplements should not be considered before ruling out this condition. Early symptoms and signs—including lethargy and fatigue—of haemochromatosis are similar to iron deficiency [11], so it is vital that this condition is not mistaken for anaemia and treated with iron supplementation. Other less common nutritional causes of anaemia, such as folic acid and vitamin B12 deficiency, should also be eliminated before considering iron supplementation.

Dietary advice is always appropriate in combating iron deficiency, and iron supplementation is indicated where the deficiency is likely to be difficult to correct by diet alone. Intensive dietary intervention has the potential to improve the iron status of young women with mild iron deficiency [12]. Although iron supplementation has resulted in more reliable improvements in iron status, both diet and supplementation have been able to improve mental health and decrease fatigue in groups of iron-deficient women [3, 13]. The Australian Institute of Sport (AIS) recognises that iron deficiency is a potential problem for athletes and that iron supplementation for female athletes with non-anaemic iron deficiency can improve some performance related parameters [14].

Good sources of dietary iron include liver, meat, beans, nuts, dried fruits, poultry, fish, whole grains or enriched cereals, soybean flour and most dark-green leafy vegetables. Iron in foods occurs in two main forms—haem as haemoglobin and myoglobin in beef, pork, poultry and fish, and non-haem, mostly as ferric salts from vegetables and dairy products [15]. Dietary haem iron is better absorbed than non-haem iron. The rate of absorption of haem iron can be assumed to be around 20% whereas absorption from various non-haem salts has a maximum level of 5–7% [15].

Oral iron supplements are generally available as ferrous salts (chloride, fumarate, gluconate, glycerophosphate, succinate, sulfate)—which are more readily absorbed than ferric salts [16]—and as iron-polysaccharide, amino acid, dextran, sorbitol, sucrose and haem chelates, and sodium ferric gluconate.

Large doses of iron (50–200 mg iron per day) are often met with poor compliance due to gastrointestinal upsets such as nausea, vomiting, diarrhoea, constipation and pain.

Succinate and sulfate salts are the most common and most easily absorbed preparations, but are more likely to cause gastrointestinal irritation, while the gluconate and fumarate salts are apparently better tolerated but not as well absorbed [17]. Fewer side effects have been reported with lower doses (less than 50 mg iron per day) and iron given as chelated, haem or controlled release elemental iron forms [18].

Controlled-release iron preparations (e.g. Novartis Slow FE: 160 mg dried ferrous sulfate equivalent to 50 mg iron; Abbott Ferro-gradumet: 325 mg dried ferrous sulfate, equivalent to 100 mg iron; Pfizer Fefol: 280 mg dried ferrous sulfate equivalent to 87 mg iron) and polysaccharide-iron complexes decrease the amount of iron released in the stomach and this may account for fewer side effects.

There are three types of controlled-release preparations. The erosion type uses an iron salt mixed with an excipient which dissolves more slowly than the iron salt. The elution type uses an inert, porous plastic matrix in which the dried ferrous sulfate is impregnated. Iron is leached from the plastic as it passes through the gastrointestinal tract and the expended matrix is excreted harmlessly in the faeces. The pelleted type consists of numerous wax- and fat-coated iron salt pellets inside a gelatin capsule. By varying the amount and type of coating, iron release can be slowed from a few minutes to several hours. The AIS recommends as suitable iron supplements Ferro-gradumet (Abbott) or Fergon elixir (Sanofi-Synthelabo): 300 mg ferrous gluconate equivalent to 33 mg iron in 5 mL.

1.4 Why investigate the iron status of female officer cadets?

The recruitment and retention of women in the ADF is a matter of concern at present, with the proportion of women in the ADF as low as 12.8% in June 2001 [19]. Defence has stated that 20% of recruits are women, so the figures suggest that retention is a problem. All employers strive to attract and retain the best people in their organisation, and the ADF is no exception. Female recruits can bring valuable skills to the ADF, which in turn cannot afford the risk of losing them due to a preventable nutritional deficiency.

1.5 Intervention study objectives

The present double-blind placebo-controlled study was designed to evaluate iron depletion as a factor in reduced physical fitness and negative psychological outcomes such as increased fatigue, in female officer cadets undertaking their first year of training at the Australian Defence Force Academy (ADFA), and to determine the effectiveness of low-dose (18 mg of elemental iron) iron supplementation in avoiding iron depletion. Because dietary iron intake, age, initial ferritin status, the presence of inflammation, usual alcohol intake and menstrual bleeding could have an effect on iron status, these factors will be taken into account when examining the effectiveness of iron supplementation. The presence of inflammation can affect concentrations of the key iron status measures making the determination of iron status difficult.

The projects aims to examine whether:

1. On average, iron status decreases over the course of physical training for cadets who do not receive an iron supplement during that course of training.
2. On average, iron status will not decrease over the course of physical training for cadets who receive a daily iron supplement of ~18mg of iron during that course of physical training.

3. Iron status amongst female officer cadets is associated with physical fitness particularly endurance, and psychological factors such as fatigue and voluntary activity, and general health.

2. Methods

2.1 Ethics approval

Before commencement of the study a member of the research team explained the purpose of the study, the risks and benefits involved, and distributed a folder containing an 'Iron Intervention Study' information sheet, ADHREC's 'Guidelines for Volunteers', a consent form (Appendix A), and the study questionnaires (Appendix B). Officer cadets who volunteered to participate in the study returned a signed consent form and completed the questionnaires. The study protocol was approved by ADHREC and all procedures of this research project conformed to the guidelines for human research ethics described in the Declaration of Helsinki (1989).

2.2 Participants and study protocol

2.2.1 Participants and exclusion criteria

The sample size requirement of 60 was based on an expected observation of a 15 ug/L difference in serum ferritin (Frt) between the treatment and placebo groups, using an estimated standard deviation of 20 ug/L, 80% power and a significance level of 0.05 [4, 20, 21]. To allow for exclusions and possible withdrawals, 100 volunteers were sought from among first-year female officer cadets. Exclusion criteria included current medical problems, recent blood donation, pregnancy in the previous 12 months, breast-feeding, anaemia (Hb <120 g/L), iron overload (Frt >300 µg/L, and/or Fe >32 µmol/L), or a positive *Helicobacter pylori* (*H pylori*) antibody test. Participants were asked to refrain from taking supplements containing iron throughout the study.

It has been reported that *H pylori* infection has presented as iron deficiency [22]. *H pylori* is a bacterium that causes a common infection of the stomach. It is estimated to be present in about 30% of Australian women [23]. Furthermore *H pylori* can decrease the effectiveness of iron supplementation [24].

Approval was not obtained to invite first-year female officer cadets at ADFA to participate; only second-year officer cadets (Army and Air Force) were available for the study. Because this population was not large enough to conduct the proposed study, female staff cadets at Royal Military College - Duntroon (RMC) were also invited to participate. This gave a possible study population of 86 female officer cadets at ADFA and 30 female staff cadets at RMC. In February 2003, 76 volunteers signed consent forms to participate in the study during the first semester of the academic year. Of these volunteers 54 had their first blood samples taken. After four volunteers were excluded because of positive tests for *H pylori* antibodies and another two volunteers withdrew, 48 volunteers were assigned to treatment or placebo group.

Because another 12 participants had withdrawn before completion of the study, a second round of the study was conducted in the second semester, which resulted in another 21 participants from ADFA (1st and 2nd year Army, Air Force and Navy officer cadets) taking part in the study. Twenty-four officer cadets assigned to the treatment group (average age

20, range 18–35 yr) and 25 assigned to the placebo group (average age 20, range 18–26 yr) completed the three blood testing points and the course of supplement/placebo capsules. Mean body mass index [BMI = wt (kg)/ht² (m)] for each group was: treatment group 22.5 (range 19–25.5) and placebo 23.0 (18.5–27.0).

2.2.2 Study protocol

Iron status measures (Haemoglobin, serum iron, ferritin, transferrin, % transferrin saturation and soluble transferrin receptor), the multistage fitness test (physical fitness), a physical activity and health diary (general health), the Multidimensional Fatigue Symptom Inventory–short form (fatigue), and the Leisure Activity Questionnaire (voluntary activity) were the factors recorded in this study. Each measure was recorded at the beginning of the academic semester (baseline), the end of six weeks (mid point) and again at 13 weeks (final point). General health was recorded by completion of a weekly physical activity diary, which included physical training sessions and any illnesses or injuries. In order to test the effect of an iron supplement on the recorded factors a double-blind placebo-controlled experimental design was chosen.

After completion of baseline blood tests, participants were divided into two groups—those above and those below the median Frt level for all participants. Participants within each group were then randomly allocated to either treatment (iron supplementation) or placebo capsules. The study was conducted in a double blind fashion with capsule codes being broken only after all measurements were completed. Participants received more than sufficient capsules for the first half semester.

To determine any effects due to menstrual blood losses or differences in dietary iron intake participants completed a food frequency questionnaire at baseline and final point and information about menstruation included in the general health and information questionnaire was used to calculate a Menstrual Bleeding Index (MBI), and hence iron losses via menstruation. Body weights were also recorded at baseline, mid point and final point in order to identify any large changes in energy expenditure or dietary intake.

Serum concentrations of C-reactive protein (CRP) were measured at the 3 time points as a marker of inflammation. Individuals with concentrations above 15 g/L are considered to have abnormally high levels and to be experiencing an inflammatory response [25]. It is known that ferritin levels rise during infection and given that CRP is a measure of inflammation during infection it is inferred that high levels of CRP indicate the presence of infection. The possible effect of CRP on the measures of iron status was also investigated as a confounding factor.

To allow estimation of compliance, participants were requested to return their coded bottles containing the remaining capsules. Bottles containing treatment/placebo capsules were reissued after six weeks. Dietary iron intake over the 13 weeks was estimated by use of a food frequency questionnaire that was completed at the final testing point.

2.3 The iron supplements

Because iron supplementation is not without risk—including gastric upset, with high doses possibly causing toxicity even in persons who are not genetically predisposed [26, 27]—we chose a low-dose iron preparation of ferrous gluconate. The Food Standards Agency (UK) Expert Group on Vitamins and Minerals states that a daily supplement of 17 mg ferrous iron would not be expected to produce adverse effects in the majority of

people, other than those with haemochromatosis [28]. Folic acid was added to the capsules to ensure good folic acid status of participants and to minimise the risk of folate-related anaemia during the study. Dietary iron intake and menstrual bleeding were taken into account when examining the effectiveness of the iron supplementation.

All capsules, which were manufactured by Gold Coast Laboratories Pty Ltd (Burleigh Junction, Qld, Australia) contained 0.5 mg folic acid (range 0.465–0.535 mg). The treatment capsule also contained 18 mg iron as ferrous gluconate (range 16.7–19.4 mg), while the placebo contained glucose. The iron and folic acid contents of the capsules were analysed by a NATA accredited analytical laboratory.

2.4 Biochemistry measurements

2.4.1 Blood collection

All participants reported to the Canberra Area Medical Centre at the same time of day (between 0630 and 0830 h) after an overnight fast. Blood from each participant was collected from a superficial antecubital vein into an EDTA tube (5 mL, BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ, USA) and a plain serum tube which contained clot activator and a gel separator (9.5 mL, Vacutainer SST Gel & Clot Activator, Becton Dickinson, Franklin Lakes, NJ, USA).

2.4.2 Haematology and *H pylori* antibody and iron status measurements

H pylori IgG antibodies were detected in fasted serum at the baseline by use of a commercial enzyme-linked immuno assay (DTect ELISA, Diagnostic Technology Pty Ltd, Belrose, NS –under licence from the School of Microbiology and Immunology, Uni of NSW, Sydney).

A basic haematological profile (haematocrit, Hct, haemoglobin concentration, Hb, red and white cell counts and counts of neutrophils, monocytes and lymphocytes) was performed on whole EDTA blood within 10 hr of collection (3 time points) by routine methods (Beckman Coulter MaxM automated analyser, Miami, Florida, USA).

Samples collected from the three time points were batch analysed, ensuring that each participant's samples were analysed within the same day. Reagents from the same production batch were used throughout the analysis. Standard commercial methods were used in the analysis of all blood analytes: Frt, high-sensitivity C-reactive protein (CRP), soluble transferrin receptor (sTfR) and transferrin (Tf) (ProSpec autoanalyser, Dade Behring, Marburg, Germany) and serum iron (Fe) (Cobas Bio clinical analyser, Roche, Germany).

Iron status was determined by measurement of Hb concentration in whole blood and Fe, Frt, sTfR and Tf concentrations in serum as well as the calculated value, transferrin saturation (TS). CRP was also measured to monitor the presence of inflammation or acute-phase proteins. The percent saturation of transferrin was calculated as:

$$TS = [\text{Fe } (\mu\text{mol/L}) / (25 \times \text{Tf (g/L)})] \times 100\%$$

Iron deficiency can be classified into three stages depending upon severity: iron depletion (ID), with decreased Frt reflecting loss of iron stores; iron-deficient erythropoiesis (IDE) indicated by a further decrease in Frt concentration and/or an increase in sTfR

concentration and decreased TS; and finally a significant decrease in circulating Hb indicating iron-deficiency anaemia (IDA). These stages are illustrated in Table 1, along with values reflective of iron excess.

Table 1: Stages of iron deficiency

Measurement	Normal	Stage 1 ID	Stage 2 IDE	Stage 3 IDA	Iron excess
Ferritin (ug/L)	30–250	< 30	<1 5	< 15	> 300
Transferrin (g/L)	2.1–3.6	Normal	> 3.6	> 3.6	
Iron (umol/L)	8–30	Normal	< 8	< 8	> 32
Transferrin Saturation (%)	16–49	Normal	< 16	< 16	≥ 55
Soluble Transferrin Receptor (mg/L)	0.4–1.8	Normal	> 1.8	> 1.8	
Haemoglobin (g/L)	120–180	Normal	Normal	< 120	

2.5 Multistage fitness test

This test, commonly referred to as the ‘shuttle run’ or ‘beep test’, was conducted as an indicator of the participants’ aerobic capacity and endurance [29]. Participants were required to run back and forth between two lines 20 m apart at progressively faster speed till volitional exhaustion. The tests were conducted at baseline, mid point and final point under the supervision of a physical training instructor during the officer or staff cadets’ usual physical training classes. Results were presented as the stage reached before volitional exhaustion (ie the higher scores indicate better a better fitness level).

2.6 Physical activity and health diary

A weekly activity and health diary (see Appendix B) was completed by participants. Any illnesses were recorded under the categories of upper respiratory tract infection, gastrointestinal upset, bleeding or bruising, muscular injury, rashes/sores, headaches, allergic reactions, influenza or other. Participants recorded whether they had experienced any of the health problems during the week. They also kept note of the hours of physical training conducted on duty.

2.7 Multidimensional Fatigue Symptom Inventory–short form

Historically, fatigue has been viewed as unidimensional with the result that researchers have focused on the severity or intensity of symptoms *per se*. However, it is now recognised that fatigue is a multidimensional phenomenon, which encompasses physical, affective, social and cognitive symptom domains [30]. The multidimensional fatigue measure, the Multidimensional Fatigue Symptom Inventory–short form (MFSI-SF) was included in this study to provide a profile of the physical, emotional, mental, vigour and general fatigue domains.

The MFSI-SF is a validated questionnaire that is sensitive enough to detect changes in fatigue over short periods [31]. It comprises 30 items; participants rate their experience of each symptom as *not at all*, *a little*, *moderately*, *quite a bit* or *extremely*. The item scores

combine to produce five subscales measuring different dimensions of fatigue—general fatigue, physical fatigue, emotional fatigue, mental fatigue and vigour (see Appendix B).

2.8 Leisure Activity Questionnaire

A Leisure Activity Questionnaire (LAC-Q, Appendix B) was used to obtain a score of leisure time activity. It asked participants to note the amount of time they spent on leisure time activities, in various categories over the previous three days. The categories were self maintenance, demanding sports, medium level sports, low level sports, social, solitary hobbies, personal improvement, relaxation, sleeping/resting, and purposeful. Each category is assigned a rating that relates to the energy expenditure. Examples are given in each category, including exercise, social and household activities. From this questionnaire a score representing level of leisure time activity per day was obtained by multiplying total minutes by an energy rating.

2.9 Anthropometry

The methods for recording anthropometric data were based on those of the International Society for the Advancement of Kinanthropometry [32]. Body weight and stature were measured at the same time of day during the blood collection sessions (i.e. three testing points). Participants were in a well-hydrated state.

2.10 Menstrual Bleeding Index

The MBI was calculated using the length of menstrual cycle (cycle length), usual length of menstrual bleeding (bleeding length) and intensity of menstrual bleeding (bleeding intensity) according to the following formula:

$$\text{MBI} = \text{bleeding length (days)} * \text{bleeding intensity (rating)} / \text{cycle length (days)}$$

where intensity is a perceived 3 point rating of 1=light, 2=medium, and 3=heavy.

This method is a modified version of that used in a previous study on iron status in female endurance athletes [33].

2.11 Food Frequency Questionnaire

Cadets' usual dietary intakes were estimated by use of a food frequency questionnaire (FFQ) developed by the Anti-Cancer Council of Victoria. This is a 96-item questionnaire that determines usual eating patterns. It has been validated and performs well compared to other methods used in this area [34]. Scanning and analysis of FFQ forms was conducted by the Anti-Cancer Council of Victoria, with data in the form of nutrient intakes and food serves returned to DSTO-Scottsdale. A copy of the questionnaire can be found at Appendix B.

No measurement of dietary intake is error free, with tendencies to underestimate nutrient intakes. The FFQ used in this study was designed to assess iron intake, and was validated against seven day weighed food records. Variation between the two methods for estimated iron intake was reported as 1.9%.

2.11.1 Treatment of dietary data

Goldberg et al [35] suggested that a ratio of energy intake (EI) to Basal Metabolic Rate (BMR) of 1.2 represents the lower 90% confidence limit for a plausible level of energy intake in relation to BMR when derived from a seven-day food record for an individual. Participants reporting energy intakes less than 1.2 of calculated BMR and participants who had completed either of the FFQs incorrectly were excluded from dietary data analyses. After volunteer withdrawals and exclusions for implausible energy intakes (11 for each testing point) only 38 pairs of FFQs were included in the data analysis. An analysis of the nutritional adequacy of the reported dietary intakes is provided at Appendix C.

2.12 Statistical Analyses

Analyses were performed by a consultant statistician. A full report is available upon request. To seek evidence of iron depletion during the study, separate analyses were applied to the iron status (biochemistry) data. Repeated-measures analysis was employed using the three responses (initial, mid-term, and final) for each subject, and polynomial contrasts were used to estimate the rate of depletion, if any, and to determine if the rate of change was the same for the first and second half of the semester. Where necessary, adjustment was made for variations among participants in dietary intake of iron, age and menstrual bleeding by including the factor as a covariate in the analysis of data at a fixed point in time.

Comparison of changes in iron status between the treatment and placebo groups was undertaken using repeated-measures analysis applied to each of the iron status variables. Additional analyses were conducted to test whether the effect of the supplement, if any, varied between the 'high' and 'low' iron status groups (based on initial Frt concentrations). Age and baseline Frt concentration were included as covariates.

To explore the manner in which the six variables in Table 1 collectively indicate iron depletion, a multivariate analysis of variance was undertaken based on the difference between initial and final measurements for each subject. The analysis was used to compare the changes in the supplement and placebo groups. Additionally, logistic regression analysis was used to compare the proportions of subjects in the supplement and placebo groups who show a decrease from the 'normal' level to a lower level (as defined in Table 1). In this analysis age was employed as a covariate. Histograms based on stages of iron deficiency in Table 1 were used to provide simple descriptive comparisons of changes over the course of the study, and comparisons between the supplement and placebo groups.

Evidence that the rate of change in fitness levels over the period of the study is different between the supplement group and the placebo group was sought by applying repeated-measures analysis to data from the fitness variable (multistage fitness test). Initial fitness level was employed as a covariate to adjust for differences in initial fitness among subjects.

Additionally, an alternative analysis was used to explore the relation between fitness level and iron depletion. Regression analyses were employed using (independently) each of the six variables in Table 1 to measure iron depletion. The analyses involved (i) the regression of change in fitness between initial and mid-term time points on change in iron status over the same period, and (ii) the regression of change in fitness between initial and final time points on change in iron status over the same period. The model allowed for differences in the relation between the supplement and placebo groups.

The same forms of analysis as indicated above for fitness were employed for the five subscales of fatigue measurement and the leisure activity measurement.

2.12.1 Inflammation as an interfering factor in the interpretation of iron status

General analysis of iron variables in this report ignored the possible effect of inflammation. However consideration was given to how the results change if participants with CRP concentrations greater than 15 g/L were excluded. There were no participants with high CRP at each of the baseline, mid point and final point. Six participants had high CRP at baseline, 1 at the mid point and 1 at the final point. There was no change in any of the findings relating to the effect of supplement or iron status.

3. Results and Discussion

3.1 Study Compliance – iron supplementation

Compliance with the daily capsule supplement was not perfect. Some volunteers ceased taking the supplement entirely and others regularly forgot to take their daily capsule. Mean compliance (n = 49), excluding those who ceased entirely, was about six days per week or 85% (range 52%–100%) which corresponded to a mean supplementation of 16.8 ± 2.1 mg (range 12.8–19.4 mg, n = 25, Figure 1).

Table 2 details the total mean daily iron and folate intake from supplement and dietary sources. It was observed that those in the placebo group with the highest dietary iron intakes had intakes similar to those in the treatment group with the lowest dietary intakes. It is conceivable that there may be some overlap in total iron intakes between the placebo and treatment groups when bioavailability of the iron is considered.

Table 2: Mean daily intake of iron and folate by female officer cadets from supplement and dietary sources

	Treatment Group N = 24		Placebo Group N = 25		F	p
	Mean \pm SD	Range	Mean \pm SD	Range		
Iron (mg)	27.5 \pm 4.0	19.5–37.6	12.7 \pm 4.8	3.0–19.6	137.6	<0.001
Folate (μ g)	642 \pm 82	504–853	648 \pm 106	415–838	0.05	0.83

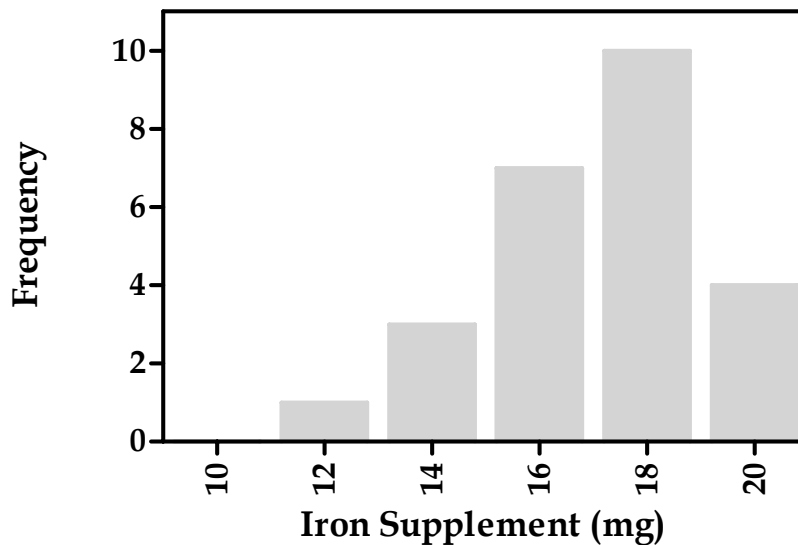


Figure 1. Iron supplementation of female officer cadets - frequency distribution. (n=25)

3.2 Were there changes in measures of iron status over time?

For all iron status measures except haemoglobin there was evidence of changed concentrations over time (Table 3). For Frt and Tsf there was evidence of a reduction in the first stage, i.e., initial to midpoint. For Frt the reduction in the first stage is reversed in the second stage, so there is no evidence of difference in levels between initial and final stages. For Tf, serum iron, TS, and sTfr there was evidence of a difference between initial and final stages with Tf and sTfr declining while serum iron and TS increased.

One possible explanation of these data is that iron was mobilised to the tissues at the expense of iron stores (Frt), most likely in response to a high level of physical activity early in the semester (Section 3.5). By the final blood testing point iron stores had been replenished, with improvements seen in Frt, sTfr and TS.

Table 4 presents the mean values for individual iron status measures for the treatment and placebo groups. There was no evidence that the apparently better iron status measures seen in the treatment group were significant.

Table 3: Mean values for individual iron status measures for all female staff and officer cadets.

	Baseline		Midpoint		Final Point	
	Mean \pm SD	Range	Mean \pm SD	Range	Mean \pm SD	Range
	N = 76		N = 56		N = 49	
Ferritin ($\mu\text{g/L}$)	34.9 \pm 22.2*	3.5–143.0	26.6 \pm 17.0*	3.1–108	27.3 \pm 16.8	4.7–82.1
C-Reactive Protein (mg/L)	4.5 \pm 8.9	0.2–45.4	2.7 \pm 4.0	0.4–24.5	2.5 \pm 3.0	0.2–15.4
Transferrin (g/L)	2.9 \pm 0.5*	1.7–4.1	2.7 \pm 0.5*	1.8–3.9	2.5 \pm 0.7*	0.9–3.7
Soluble Transferrin Receptor (mg/L)	1.5 \pm 0.3*	0.9–3.0	1.5 \pm 0.4	0.7–3.1	1.3 \pm 0.4*	0.4–1.9
Transferrin Saturation (%)	23.3 \pm 9.4*	5.8–54.8	26.7 \pm 11.7	6.7–57.1	38.9 \pm 25.2*	8.6–171.8
Haemoglobin (g/L)	138 \pm 9	118–157	137 \pm 7	121–153	139 \pm 8	119–156

For all variables except haemoglobin the overall change in concentration over time ($n = 49$) was significant. Significant difference between baseline and midpoint and baseline and final points are indicated (*).

Table 4: Mean values for individual iron status measures for female staff and officer cadets in the treatment and placebo groups^a

	Baseline		Midpoint		Final Point	
	Placebo	Treatment	Placebo	Treatment	Placebo	Treatment
	n = 25	n = 24	n = 25	n = 24	n = 25	n = 24
Ferritin ($\mu\text{g/L}$)	28.9	32.2	22.4	26.6	24.1	30.7
Age adjusted	29.1	31.4	23.2	25.7	25.0	29.5
Transferrin (g/L)	2.87	2.90	2.76	2.68	2.49	2.41
Soluble Transferrin Receptor (mg/L)	1.49	1.46	1.51	1.46	1.32	1.18
Transferrin Saturation (%)	25.1	24.7	26.1	28.2	33.6	38.5
Age adjusted	25.1	24.2	25.6	30.2	33.2	39.2
Haemoglobin (g/L)	140	137	138	137	139	138

^aWhere age is a significant covariate, age adjusted means are included. The values are adjusted to those expected for participants with an age of 20.3 years. There was no evidence that the apparently better iron status measures seen in the treatment group were significant.

3.3 Prevalence of iron deficiency

For those cadets who had iron status measured at each testing point, there was no evidence for change in the prevalence or severity of iron deficiency between the baseline, midpoint and final test points. The percentage of female officer and staff cadets with individual iron status measures outside the normal reference ranges is presented in Table 5 and the prevalence of iron deficiency with time is shown in Figure 2.

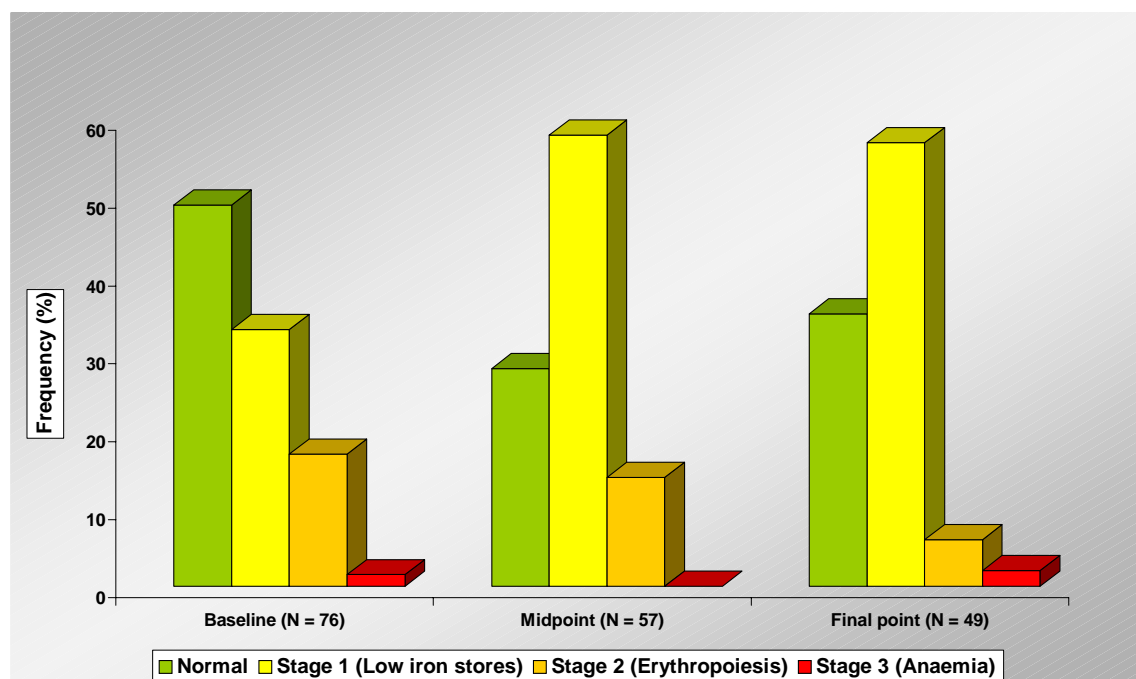


Figure 1. Prevalence of iron deficiency. The frequency distribution includes the results of those female officer and staff cadets who withdrew from the study (i.e. did not present for the next blood testing point). Criteria for classifying iron deficiency are in Table 1. ID = iron deficiency or stage 1; IDE = iron deficiency erythropoiesis or stage 2, IDA = iron deficiency anaemia or stage 3. There was no evidence for changes in prevalence and severity of iron deficiency between testing points or between treatment and placebo groups. Treatment group = 24, Placebo group = 25 and Withdrawn = 27.

3.4 Does alcohol, dietary iron intake, initial iron status or inflammation influence the effect of the supplement?

3.4.1 Alcohol:

The average daily alcohol consumption was equivalent to 1.5 standard drinks (~15 g alcohol) per day and ranged from only one or two drinks per week to 35 drinks per week. Although the FFQ did not record the drinking pattern, it is likely that most of the alcohol was consumed over weekends. Alcohol was included as a possible factor affecting iron status in the following statistical analyses.

Table 5: Number (and percentage) of female staff and officer cadets with iron status measures outside normal iron status cut-off values.^a

	Baseline n = 76	Midpoint n = 56	Final Point n = 49
Ferritin (µg/L)	43 (56.5%)	50 (89%)*	43 (88.0%)
Transferrin (g/L)	8 (10.5%)	2 (3.5%)*	2 (4.1%)*
Soluble Transferrin Receptor (mg/L)	9 (12%)	11 (19.5%)	2 (4.1%)*
Transferrin Saturation (%)	16 (21%)	8 (14.5%)	4 (8.2%)*
Haemoglobin (g/L)	1 (1.5%)	0 (0%)	1 (2%)
C-Reactive Protein (mg/L)	11 (15%)	8 (14.5%)	5 (11%)

^a Elevated concentrations of C-reactive protein indicate the presence of inflammation. Significant difference between baseline and midpoint and baseline and final points are indicated (*).

There was evidence of a strong negative relationship between changes in haemoglobin concentration from initial to final testing and usual daily alcohol consumption recorded at the initial point ($p = 0.007$). Generally, the heavier drinkers were more likely to have decreased concentrations of haemoglobin at the final testing point.

There was also evidence of a strong negative relation between usual daily alcohol consumption in the previous 12 months and baseline transferrin saturation. Female staff and officer cadets who consumed on average more than about 15 g of alcohol per day (1.5 standard drinks) were highly likely to have low transferrin saturation. This was principally a result of the effect of alcohol on plasma iron concentrations, as indicated by the strong negative relationship between alcohol consumption and plasma iron concentrations. The effect was not evident for transferrin.

This is an unusual association given that alcohol is a disruptor of iron homeostasis, which results in increased absorption of iron, particularly haem iron [36]. Furthermore, alcohol, haem iron and iron supplements have been shown to be dietary determinants of plasma Frt [37], so the expected effect of a high consumption of alcohol would be increased serum iron and increased serum ferritin concentration.

A feasible explanation of the present situation might relate to the behaviour associated with alcohol consumption. For example, 'binge' drinking on Friday and Saturday nights could be associated with poor eating habits over the weekend and a disordered eating pattern during the week. Such a diet might contain less bioavailable iron. A more detailed study of dietary intake would be required to test this proposition.

To determine if the effect of the supplement might depend on the usual average daily alcohol consumption alcohol intake was used as a covariate in the analysis of change from initial to midpoint and from initial to final time of measurement. For no variable was there evidence of a difference between mean iron levels in the supplemental and placebo groups after adjustment for differences in initial alcohol level.

3.4.2 Dietary iron intake:

To determine if the effect of the supplement might depend on prior dietary iron intake the variable iron1 was used as a covariate in the analysis of change from initial to midpoint and from initial to final time of measurement. For no variable was there evidence of a difference between the supplement and placebo groups in respect of changes in iron status variables after adjustment for differences in initial level of dietary iron.

The statistical analysis was repeated with the usual daily dietary iron intake during the study being used in place of the usual daily dietary iron intake in the 12 months prior to the study. The findings were the same.

3.4.3 Initial measures of iron status:

To determine if the effect of the supplement might be related to initial iron status the initial serum Frt concentration was used as a covariate in the analysis of change from initial to midpoint and from initial to final time of measurement. Negative changes in plasma ferritin concentrations became greater as the baseline ferritin concentration was higher. There is no difference between the supplement group and the placebo group whether Frt levels were initially high or low. (This is judged by comparing the slopes of the lines fitted to the treated and placebo groups – if the treatment were maintaining iron levels then the slope of the line for the treated group should be less than that for the placebo group.)

The change in serum Tsf concentrations from initial to final points was related to initial Frt concentrations with increases being more likely when initial Frt concentrations were lower. For the other four iron variables examined there is no relation with initial ferritin levels.

These findings are consistent with iron being more easily mobilised from a liver with greater iron stores. This was also the finding in a recent study which investigated the iron cost of a 12-week weight-training program [38].

3.5 Were there changes in fitness, fatigue, leisure activity or general health over time?

Leisure activity and health scores did not change over time, and apart from the general fatigue score, for which there was a decrease ($F = 5.83$, $p = 0.02$), fatigue scores did not change.

There was evidence that the multistage fitness resulted improved at the midpoint ($p < 0.001$) and that this increase in fitness was lost by the final test point. However the difference in average level of fitness between the supplement and placebo groups, which was evident at the individual times, was not large enough to be significant (Figure 3). There was no evidence of a relation between either the initial fitness result or the change in fitness results and any of the iron status measures. Neither was fitness related to fatigue or leisure activities. There was no evidence that alcohol consumption, whether prior to or during the study, affected changes in fitness over the time of the study, nor was there evidence that alcohol consumption had different effects on the supplement and placebo groups.

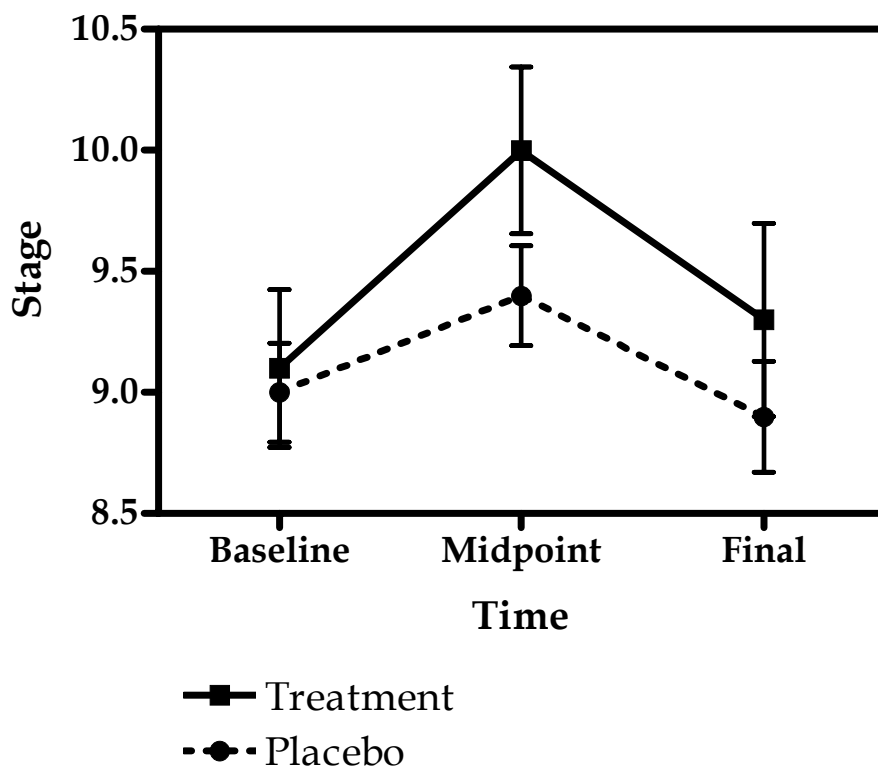


Figure 3. Multistage Fitness Test. There was evidence that the multistage fitness resulted improved at the midpoint ($p < 0.001$) and that this increase in fitness was lost by the final test point. However the difference in average level of fitness between the supplement and placebo groups, which was evident at the individual times, was not large enough to be significant.

Leisure activity scores, which relate to minutes per day times a multiplier for physical effort, remained stable throughout the semester and were similar for the treatment and placebo groups: 89 ± 34 , 100 ± 44 , 93 ± 30 for baseline, midpoint and final testing points respectively ($n = 46$).

The MFSI-SF results are presented in Table 6. The level of general fatigue at baseline was fairly high and appears to reflect the higher level of physical activity during the early weeks of the semester. Other fatigue dimension scores were within expected levels for university study with low-moderate levels of physical training.

There was no evidence that the improvement in the general fatigue score was associated with improvement in any of the iron status measures. However, there was some evidence that the dimension of emotional fatigue might be sensitive to iron treatment. A recent iron intervention study involving young Australian women [3] found measures of general health and fatigue to respond positively to a high dose iron supplement (105 mg iron), although measures of perceived physical health did not respond similarly. It may be that emotional fatigue is particularly sensitive to iron status [30].

University contact hours and time spent in organised physical training varied greatly between RMC and ADFA, between students and weeks, but not between the treatment and placebo groups. For example, in the six weeks when there were few or no formal classes, physical training was conducted for 30 to 42 hours. Some students had a greater number of hours in formal classes each week – up to 47 hours – and spent fewer hours in physical training. Mean formal class attendance per week over the 12 weeks totalled 13.3 h (range 0–47.5 h) and mean time spent in physical training was 10 h per week (range 0–42 h) for the two groups (Figure 4).

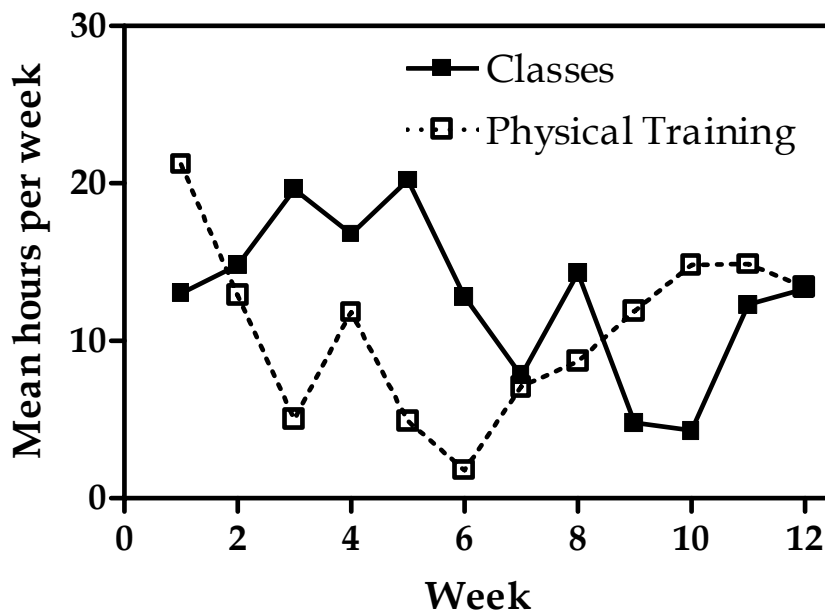


Figure 4. Mean hours per week spent in formal classes and physical training by female officer and staff cadets (N = 49).

Table 6: Multidimensional Fatigue Symptom Inventory (Short Form) scores for female staff and officer cadets.^a

	Baseline		Midpoint		Final Point	
	Placebo	Treatment	Placebo	Treatment	Placebo	Treatment
	N = 23	N = 23	N = 23	N = 23	N = 23	N = 23
General fatigue	17.2 ± 4.6	13.6 ± 4.6	16.1 ± 4.7	11.6 ± 3.4	14.5 ± 4.4	12.0 ± 4.3
Physical fatigue	10.5 ± 3.0	9.3 ± 4.0	11.2 ± 3.1	9.1 ± 2.9	10.2 ± 2.9	9.6 ± 3.9
Emotional fatigue	11.0 ± 3.7	10.8 ± 3.5	11.1 ± 4.8	9.3 ± 3.2	11.7 ± 3.5*	9.3 ± 3.4*
Mental fatigue	12.3 ± 3.7	10.8 ± 3.5	12.1 ± 3.1	10.9 ± 4.0	11.9 ± 3.3	10.1 ± 3.3
Vigour fatigue	17.6 ± 3.5	20.3 ± 3.5	16.4 ± 4.0	20.1 ± 4.1	16.2 ± 4.2	19.3 ± 4.6

^a There was evidence that the change in the level of emotional fatigue between baseline and final testing points differs for the two groups ($p = 0.01$) with the treatment group showing a decline that is not seen in the placebo group.

General health problems were not related to iron status or any other variables measured during this study. Table 7 gives a summary of the health problems recorded over the 12 weeks. The most common complaint was muscle soreness.

Table 7: Summary of weekly health problems recorded by female staff and officer cadets^a

	Upper respiratory Tract infection	Gut upsets	Minor trauma	Muscular injury or soreness	Skin rashes or sunburn	Head ache	Allergic reaction	Influenza	Other
No.	84	10	31	100	7	61	1	32	2
%	26%	3%	9%	30%	2%	19%	0%	10%	1%

No. refers to the total number of recorded incidences for the health problem during the 13 weeks. The total possible number for each health problem for 49 staff or officer cadets over 13 weeks was 637.

3.6 Was adjustment needed for variations among participants in age, dietary intake of iron or menstrual bleeding?

3.6.1 Age:

The supplement group was over-represented in the under-20 age group and under-represented in the 20 to 26 year old age group. This should not influence the effect of iron status or supplement over time between the treatment and placebo groups because each subject is acting as their own control in this regard. However, in the case of TS and sTfR there was a relation between the change in iron levels across time and age, i.e., the age-dependence was not consistent at each point. Tests were repeated for these comparisons using age as a covariate but did not alter the conclusions.

3.6.2 Iron intake:

There was no evidence that the officer and staff cadets altered their eating habits during the study or that the treatment and placebo groups ate differently (paired t tests, $p > 0.05$). Consistent with the unchanged eating habits were the stable body weights recorded by officer and staff cadets throughout the semester (mean of 65 kg, range 51.0–82.5 kg). Covariate adjustment for dietary iron intake was not required in subsequent data analyses.

A high proportion of participants were at risk of not eating sufficient of one or more nutrients to meet their requirements (see Appendix C). About one-third of the participants were at risk of eating insufficient dietary iron to meet their nutritional requirements (RMDI) in the year preceding the study. The mean total iron intake for the treatment group was 27.5 mg (range 19.5–37.6 mg) and for the placebo group was 12.7 mg (range 3.0–19.6 mg). Although no association was found between usual iron intake and initial measures of iron status, evidence was found that the change in the F_{rt} level between the initial and midpoint observations was related to the initial dietary iron level ($p=0.02$) i.e. cadets with the lowest dietary iron intake were most likely to have a decrease in serum F_{rt} during the first half of the study.

3.6.3 Menstrual bleeding index:

The relation between level of menstrual bleeding and initial iron levels, as measured by the 6 iron status variables, was examined. There was found to be no significant correlation between MBI and any of the variables defining initial iron levels.

To determine if the effect of the supplement might be related to the level of menstrual bleeding the variable MBI was used as a covariate in the analysis of change from initial to midpoint and from initial to final time of measurement. For no variable was there evidence of a difference between mean iron levels in the supplemental and placebo groups after making allowance for different levels of menstrual bleeding. Additionally a test was performed to determine if any change in iron status is related to the level of menstrual bleeding. For no variable is there evidence that the change in iron status was dependent on the level of menstrual bleeding.

4. Summary and Conclusions

4.1 Intervention study objectives

4.1.1 On average, iron status changed over the course of physical training for cadets who did not receive iron supplementation during the course of physical training.

The iron status measures indicated that iron was mobilised to the tissues at the expense of iron stores, most likely in response to a high level of physical activity early in the semester. In the second half of the semester iron stores were replenished, with improvements seen in all iron status measures. The prevalence of iron deficiency among the group was higher than reported for national surveys of this age group. More than half of the young women commenced the study with iron deficiency to some degree and ~6% had iron-deficiency anaemia. The prevalence of iron deficiency remained the same at each testing point. Although as a group, there was no evidence of net decline in iron status, some individuals did not fully recover their iron stores in the second half of the semester.

4.1.2 On average, iron status was not affected over the course of physical training for cadets who received a daily iron supplement of ~18mg of iron during that course of physical training.

4.1.3 Iron status amongst female officer cadets was not associated with physical fitness, fatigue and leisure activity, and general health.

There was no evidence for any differences between the treatment and placebo groups in the patterns of change for any of the biochemical, dietary, physiological or behavioural measures. This conclusion held after adjustment for age, level of menstrual bleeding, baseline plasma ferritin concentrations, history of dietary iron intake and alcohol intake. With one exception, there was no difference in pattern of change in the behavioural variables (fitness, fatigue or leisure activities) between treatment and placebo groups. The one exception was emotional fatigue, where there was a decreased score over time for the treatment group, which was not evident in the placebo group. Emotional fatigue might be a fatigue dimension that is particularly sensitive to iron status.

There is a small 'iron cost' associated with increased exercise and the ability of the officer and staff cadets to cope would largely depend on having good iron stores before commencing physical training, because it is unlikely that they would make up the shortfall quickly through usual diet alone [39]. The decrease in iron stores had no apparent effect on physical performance. In fact there was a mean increase in aerobic endurance for this cohort at the mid-semester testing point. Most participants apparently achieved a new

steady-state with lower iron stores, but with adequate tissue levels of iron. The risk is that officer and staff cadets may become progressively iron deficient as a result of repeated physical training programs or deployments. DSTO-Scottsdale has shown that the iron status of soldiers declines over the course of routine military field exercises [7, 40, 41]. It has now also been shown that a low-dose iron supplement is not likely to prevent such a decline in iron status, at least in women.

General health and levels of fatigue were not problematic and remained stable across the semester. The level of general fatigue was relatively high at baseline, reflecting the higher physical demands of training early in the semester. The decreases in general fatigue observed at the middle and end of the semester were not associated with iron status.

Many of the female staff and officer cadets drank in excess of the Australian guidelines for safe alcohol consumption. The mean daily intake was equivalent to 1.5 standard drinks (~15 g alcohol) and ranged from only one or two drinks per week to 35 drinks per week. Although the FFQ did not record the drinking pattern, it is likely that most of the alcohol was consumed over weekends. This drinking pattern puts the staff and officer cadets at risk of iron deficiency, in terms of both low haemoglobin concentration and low transferrin saturation. Poor eating habits associated with 'binge' drinking is one possible explanation of this observation.

5. Recommendations

1. Good dietary advice is always appropriate for the prevention and treatment of iron deficiency. It is recommended that a nutrition education program be introduced at both RMC and ADFA. This program should highlight the need for female staff and officer cadets to choose good sources of iron in their daily diet, and to consume alcohol at no more than moderate levels. A simple program could be designed, implemented and evaluated by DSTO nutritionists in collaboration with the Canberra Area Medical Centre's dietitian and with advice from relevant ADF instructors. Such a program would most likely involve promotion of good nutrition messages within the medical centre, mess, gymnasium and residences and could also involve inclusion of study material within any relevant courses.
2. Iron status of females should be monitored at ADFA and RMC. Female staff and officer cadets are at high risk of iron deficiency and anaemia. Iron deficiency is a preventable/treatable medical condition, which can have an adverse effect on performance and health. Screening of female staff and officer cadets for iron deficiency should be considered. An appropriate minimum screening schedule would be the measurement of serum ferritin on entry to officer training and then as a two-year follow-up. However, officer or staff cadets who complain of unusual tiredness/fatigue and whose aerobic endurance (i.e. shuttle run or 2.4-km run times) decline or fail to improve despite regular PT should have their iron status investigated. During periods of intense physical training, cadets may have elevated ferritin due to inflammation. Therefore an investigation of iron status during this period should include C-reactive protein. In the case of an elevated C-reactive protein level, the iron investigation should be repeated at a later date when the inflammation has subsided.

3. Suspected cases of iron deficiency (and iron overload) should be referred for medical attention. Self-administration of iron supplements—even low dose—is not recommended. Supplemental iron—preferably a controlled-release form providing 30–100 mg of iron per day—should be administered only under medical supervision.

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7. Acknowledgements

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Appendix A: Information & Consent Forms

INFORMATION SHEET IRON INTERVENTION STUDY

Iron deficiency is the most common nutrient deficiency in the world, affecting approximately 12.5% of Australian women between the ages of 15 and 30. Young women are at risk of iron deficiency because of low dietary intake, and a higher iron requirement created by growth, puberty and the onset of menstruation.

Iron is required for the synthesis of haemoglobin, a blood protein which is the oxygen transport component of blood. Iron is also involved in biological activities related to immunity, cognitive functioning (the ability to think clearly) and thermoregulation (the regulation of body temperature). Iron deficiency can have negative effects on physical performance, even in mild iron deficiency. Other symptoms such as fatigue, lethargy, weakness, headaches, irritability and dizziness have been associated with iron deficiency. These symptoms can affect general health, and reduce the quality of life in terms of work, leisure, sports performance, social activities and family responsibilities.

You may be at increased risk of iron deficiency as your physical activity increases during your ADF training and the tiredness often associated with iron-deficiency could add to the difficulty of your training program and make you feel less like participating in social activities.

Because the skills that you bring into the Defence Force are valued, the ADF doesn't want a preventable nutritional deficiency, such as iron deficiency, preventing you from achieving your best during your physical and academic training. This study aims to determine the extent of iron deficiency among the women in your year and to determine whether or not a low dose iron supplement can prevent some of the symptoms associated with iron deficiency.

Your Part in the Study

Participation in the study is entirely voluntary; there is no obligation to take part in the study, if you choose not to participate there will be no detriment to your career or future health care. You may withdraw at any time with no detriment to your military career or future health care.

In this study you will be expected to:

1. Complete a general health and information questionnaire (GHI), and have your weight measured;
2. Complete the remaining questionnaires (FAT-Q, LAC-Q, FFQ). You will be asked to complete these questionnaires on three occasions during the twelve week study;
3. Donate a blood sample on three occasions during the study, for the purpose of determining your iron status;
4. Complete an endurance test, on three occasions, which involves a 2.4km run;
5. Keep an activity diary noting physical training you have done and any health problems you have experienced; and
6. Take a supplement every morning, with citrus juice, and not to drink milk until after breakfast, for the twelve weeks of the study.

By participating in this study you are giving an undertaking that you are not currently being treated by a medical practitioner for any medical condition, that you are not pregnant, have not been pregnant in the last 12 months, and are not trying to become pregnant.

Risks of Participation

Although the supplement you receive is a low dose nutritional supplement, there is a small risk that you might have a reaction to it (for example wind, or constipation). If you are concerned that the supplement is causing serious discomfort, you should contact the researchers immediately.

The risks involved in having a blood sample taken are no higher than they are for a routine medical examination. An experienced phlebotomist will place a tourniquet around your upper arm then draw a blood sample from the inside of your arm into a syringe. Apart from a small prick when the needle pierces the skin, little discomfort is experienced by most people. However, if the procedure makes you feel faint, you should remain sitting and place your head between your knees. By applying pressure on the puncture site after the needle is withdrawn, you may prevent any bruising.

The use of sterile technique by the phlebotomist will prevent the risk of infection.

All consumables used to sample blood (eg. needle, gloves, swabs, tape) will be sterile prior to use, and the sterile technique used by the phlebotomist will prevent the risk of infection. Conventional medical practice and standards for handling human tissue will be followed in the collection and handling of blood at all times.

Benefits of Participation

Your benefits for participation in this study include assessment of your diet, and determination of your iron status. Dietary advice will be made available from an accredited practicing dietitian on completion on the study, as well as the results of the study for your information.

On Duty

Whilst involved in this study you will be considered to be 'on duty'. All of the activities will be conducted within the hours you are considered to be 'on duty'.

Statement of Privacy

The information collected during the study will only be used for this study, and will be kept confidential at all times. Forms filled out by you will be identified by a code number, and will have your name and service number removed. Only the researchers know your code number. All details and results are kept in a locked filing cabinet accessible only by the researchers. Nothing will be published which will identify individual participants..

Should you have any complaints or concerns about the manner in which this project is conducted, please do not hesitate to contact the researchers in person:

Julia Carins, DSTO-Scottsdale, 76 George St, Scottsdale, TAS 7260
Phone 03 6352 6614 Fax 03 6352 3044
email: julia.carins@dsto.defence.gov.au

Dr Christine Booth, DSTO-Scottsdale, 76 George St, Scottsdale TAS 7260
Phone 03 6352 6609 Fax 03 6352 3044
email: christine.booth@dsto.defence.gov.au

OR you may prefer to contact the Australian Defence Human Research Ethics Committee at the following address:

Executive Secretary,
Australian Defence Human Research Ethics Committee
CP2-7-66
Department of Defence
CANBERRA ACT 2600
Phone: 02 6266 3837; Fax: 02 62664982
email: ADHREC@defence.gov.au

CONSENT FORM IRON INTERVENTION STUDY

I, give my consent to participate in the study mentioned above on the following basis:

I have had explained to me the aim of this research project, how it will be conducted and my role in it.

I understand the risks involved as described above.

I understand that:

- participation in the study is entirely voluntary and there is no obligation to take part in the study;
- had I chosen not to participate there would be no detriment to my career or future health care; and
- I may withdraw at any time with no detriment to my career or to my future health care.

I am co-operating in this project on condition that:

- the information I provide will be kept confidential;
- the information will be used only for this project; and
- the research results will be made available to me at my request and any published reports of this study will preserve my anonymity.

I have been given a copy of the information/consent sheet, signed by me and by one of the principal researchers, Julia Carins or Christine Booth to keep.

Should I have any complaints or concerns about the manner in which this project is conducted I will contact the researchers in person, or contact the Australian Defence Human Research Ethics Committee at the following address:

Executive Secretary
Australian Defence Human Research Ethics Committee
CP2-7-66
Department of Defence
CANBERRA ACT 2600
Telephone: (02) 6266 3837 Fax: (02) 62664982
E-mail: ADHREC@defence.gov.au'

I have also been given a copy of ADHREC's *Guidelines for Volunteers*.

.....
SUBJECT

.....
DATE

.....
RESEARCHER

.....
DATE

FITNESS TEST RESULTS IRON INTERVENTION STUDY

I, give my permission to the researchers conducting the Iron Intervention Study to obtain any of my fitness test results from my PT instructor during the 13 weeks of the study.

The fitness test results may include:

- Number of shuttles completed in shuttle run test
- Number of push ups completed in BFA
- Number of chin ups completed in BFA
- Race times

I understand that these results will be kept confidential, will be used only for this project, will be made available to me at my request, and when reported, will preserve my anonymity.

.....
SUBJECT	DATE	RESEARCHER	DATE

APPENDIX B: Study Questionnaires

LAC-Q

Think about the things you have been doing over the **last 3 days, not counting today**. Consider the activities that you have done outside of work time, in your **leisure time**. For each activity category, note the number of times you have done such an activity, and estimate the **total time** you have spent doing that type of activity **over the past 3 days**.

Activity Category	Examples	Total time on activity	
		Hours	Min
Self maintenance	Showering, getting dressed, doing hair, eating, cooking, tidying up room, ironing, grocery shopping.		
Demanding sports	Swimming (fast), tennis (singles), squash, hockey, football (rugby, soccer, Aussie Rules), basketball (game), bicycling (hard, stationary or road), aerobics, running, rowing, circuit training, vigorous conditioning exercise (e.g. pushups, situps, chinups)		
Medium level sports	Conditioning exercise (light or moderate effort), lifting weights, easy bicycling, tennis (doubles), swimming (moderate intensity), basketball (practice), cricket, fast walking (7 km/h), golf (pulling clubs)		
Low level sports	Volleyball, billiards, gymnastics (general), table tennis		
Social	Playing cards, shopping with friends, going out for a drink, going to restaurant/movies.		
	Dancing/Clubbing		
Solitary hobbies	Drawing/painting, writing, playing musical instruments, sewing, going for a walk (slow strolling pace)		
Personal improvement	Evening classes, learning a new hobby, studying		
Relaxation	Taking a bath, reading, listening to CD's, watching TV, surfing the internet		
Sleeping/resting	Sleeping, napping, lying down for a rest		
Purposeful	Going to club/church/meeting, (low activity)		
	Club activities/volunteer work (medium/high activity)		
Other: (Please specify)			
Other: (Please specify)			

Dietary Questionnaire

*Questions about what you
usually eat and drink*

Please fill in the
date you
completed this
questionnaire:

DAY	MTH	YEAR
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>
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<input type="text"/>	<input type="text"/>	<input type="text"/>
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<input type="text"/>	<input type="text"/>	<input type="text"/>

INSTRUCTIONS:

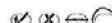
This questionnaire is about your usual eating habits over the past 12 months. Where possible give only **one answer per question** for the type of food you eat **most often**. (If you can't decide which type you have most often, answer for the types you usually eat.)

- Use a soft pencil only, preferably 2B.
- Do not use **any** biro or felt tip pen.
- Erase mistakes fully.
- Make no stray marks.

Please MARK LIKE THIS:



NOT LIKE THIS:



1. How many pieces of fresh fruit do you usually eat per day? (Count 1/2 cup of diced fruit, berries or grapes as one piece.)

- ☐ I don't eat fruit
- ☐ less than 1 piece of fruit per day
- ☐ 1 piece of fruit per day
- ☐ 2 pieces of fruit per day
- ☐ 3 pieces of fruit per day
- ☐ 4 or more pieces of fruit per day

2. How many different vegetables do you usually eat per day? (Count all types, fresh, frozen or tinned.)

- ☐ less than 1 vegetable per day
- ☐ 1 vegetable per day
- ☐ 2 vegetables per day
- ☐ 3 vegetables per day
- ☐ 4 vegetables per day
- ☐ 5 vegetables per day
- ☐ 6 or more vegetables per day

3. What type of milk do you usually use?

- ☐ none
- ☐ full cream milk
- ☐ reduced fat milk
- ☐ skim milk
- ☐ soya milk

4. How much milk do you usually use per day? (Include flavoured milk and milk added to tea, coffee, cereal etc.)

- ☐ none
- ☐ less than 250 ml (1 large cup or mug)
- ☐ between 250 and 500 ml (1-2 cups)
- ☐ between 500 and 750 ml (2-3 cups)
- ☐ 750 ml (3 cups) or more

5. What type of bread do you usually eat?

- ☐ I don't eat bread
- ☐ high fibre white bread
- ☐ white bread
- ☐ wholemeal bread
- ☐ rye bread
- ☐ multi-grain bread

6. How many slices of bread do you usually eat per day? (Include all types, fresh or toasted and count one bread roll as 2 slices.)

- ☐ less than 1 slice per day
- ☐ 1 slice per day
- ☐ 2 slices per day
- ☐ 3 slices per day
- ☐ 4 slices per day
- ☐ 5-7 slices per day
- ☐ 8 or more slices per day

7. Which spread do you usually put on bread?

- ☐ I don't usually use any fat spread
- ☐ margarine of any kind
- ☐ polyunsaturated margarine
- ☐ monounsaturated margarine
- ☐ butter and margarine blends
- ☐ butter

8. On average, how many teaspoons of sugar do you usually use per day? (Include sugar taken with tea and coffee and on breakfast cereal etc.)

- ☐ none
- ☐ 1 to 4 teaspoons per day
- ☐ 5 to 8 teaspoons per day
- ☐ 9 to 12 teaspoons per day
- ☐ more than 12 teaspoons per day

9. On average, how many eggs do you usually eat per week?

- ☐ I don't eat eggs
- ☐ less than 1 egg per week
- ☐ 1 to 2 eggs per week
- ☐ 3 to 5 eggs per week
- ☐ 6 or more eggs per week

10. What types of cheese do you usually eat?

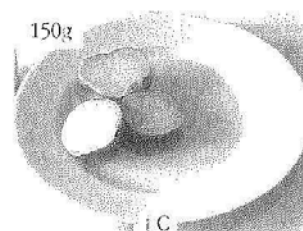
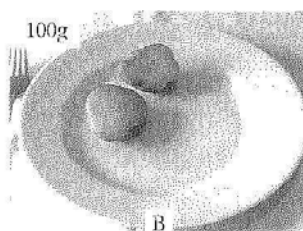
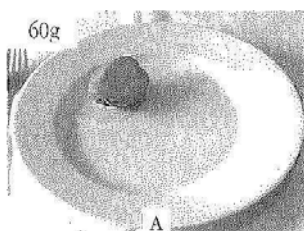
- ☐ I don't eat cheese
- ☐ hard cheeses, e.g. parmesan, romano
- ☐ firm cheeses, e.g. cheddar, edam
- ☐ soft cheeses, e.g. camembert, brie
- ☐ ricotta or cottage cheese
- ☐ cream cheese
- ☐ low fat cheese

For each food shown on this page, indicate **how much on average you would usually have eaten at main meals during the past 12 months**. When answering each question, think of the **amount** of that food you usually ate, even though you may rarely have eaten the food on its own.

If you usually ate more than one helping, fill in the oval for the serving size closest to the **total amount** you ate.

11. When you ate potato, did you usually eat:

☐ I never ate potato



☐ Less than A

☐ A

☐ Between A & B

☐ B

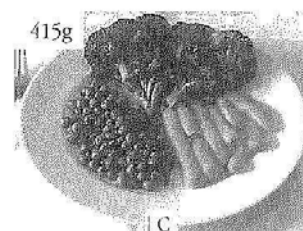
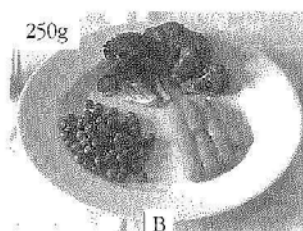
☐ Between B & C

☐ C

☐ More than C

12. When you ate vegetables, did you usually eat:

☐ I never ate vegetables



☐ Less than A

☐ A

☐ Between A & B

☐ B

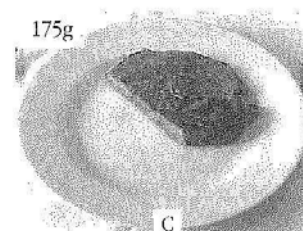
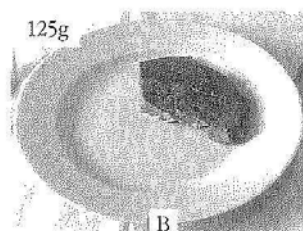
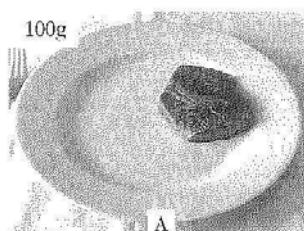
☐ Between B & C

☐ C

☐ More than C

13. When you ate steak, did you usually eat:

☐ I never ate steak



☐ Less than A

☐ A

☐ Between A & B

☐ B

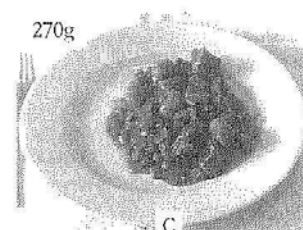
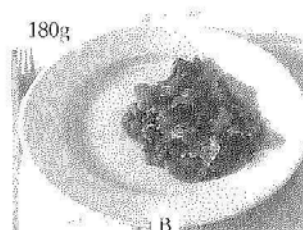
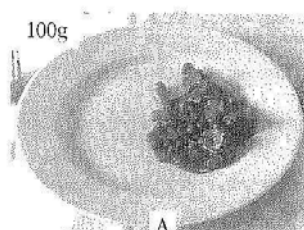
☐ Between B & C

☐ C

☐ More than C

14. When you ate meat or vegetable casserole, did you usually eat:

☐ I never ate casserole



☐ Less than A

☐ A

☐ Between A & B

☐ B

☐ Between B & C

☐ C

☐ More than C

5. Over the last 12 months, on average, *how often* did you eat the following foods? Please completely fill one oval in every line. Please MARK LIKE THIS: ☐ ☒ ☐ ☐ NOT LIKE THIS: ☒ ☒ ☐ ☐

Times You Have Eaten		N E V E R	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
			per month	per week				per day			
CEREAL FOODS, SWEETS & SNACKS											
	All Bran TM	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Sultana Bran TM , FibraPlus TM , Branflakes TM	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Weet Bix TM , Vita Bix TM , Weetites TM	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Cornflakes, Nutrigrain TM , Special K TM	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Porridge	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Muesli	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Rice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Pasta or noodles (include lasagne)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Crackers, crispbreads, dry biscuits	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Sweet biscuits	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Cakes, sweet pies, tarts and other sweet pastries	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Meat pies, pasties, quiche and other savoury pastries	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Pizza	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Hamburger with a bun	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Chocolate	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Flavoured milk drink (cocoa, Milo TM etc.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Nuts	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Peanut butter or peanut paste	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Corn chips, potato crisps, Twisties TM etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Jam, marmalade, honey or syrups	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Vegenite TM , Marmite TM or Promite TM	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
DAIRY PRODUCTS, MEAT & FISH											
	Cheese	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Ice-cream	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Yoghurt	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Beef	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Veal	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Chicken	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Lamb	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Pork	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Bacon	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Ham	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Corned beef, luncheon meats or salami	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Sausages or frankfurters	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Fish, steamed, grilled or baked	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Fish, fried (include take-away)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Fish, tinned (salmon, tuna, sardines etc.)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
FRUIT											
	Tinned or frozen fruit (any kind)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Fruit juice	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Oranges or other citrus fruit	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Apples	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Pears	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Bananas	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Watermelon, rockmelon (cantaloupe), honeydew etc.	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Pineapple	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Strawberries	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Apricots	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Peaches or nectarines	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Mango or paw paw	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	Avocado	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

<i>Times You Have Eaten</i> CONTINUED		N E V E R	less than once	1 to 3 times	1 time	2 times	3 to 4 times	5 to 6 times	1 time	2 times	3 or more times
		per month			per week				per day		
VEGETABLES (INCLUDING FRESH, FROZEN AND TINNED)											
Potatoes roasted or fried (include hot chips)											
Potatoes cooked without fat											
Tomato sauce, tomato paste or dried tomatoes											
Fresh or tinned tomatoes											
Peppers (capsicum)											
Lettuce, endive, or other salad greens											
Cucumber											
Celery											
Beetroot											
Carrots											
Cabbage or Brussels sprouts											
Cauliflower											
Broccoli											
Silverbeet or spinach											
Peas											
Green beans											
Bean sprouts or alfalfa sprouts											
Baked beans											
Soy beans, soy bean curd or tofu											
Other beans (include chick peas, lentils etc.)											
Pumpkin											
Onion or leeks											
Garlic (not garlic tablets)											
Mushrooms											
Zucchini											

16. Over the last 12 months, how often did you drink beer, wine and/or spirits?

<i>Times That You Drank</i>	N E V E R	less than once a month	1-3 days per month	1 day per week	2 days per week	3 days per week	4 days per week	5 days per week	6 days per week	every day
Beer (low alcohol)										
Beer (full strength)										
Red wine										
White wine (include sparkling wines)										
Fortified wines, port, sherry, etc.										
Spirits, liqueurs, etc.										

When answering the next two questions, please convert the amounts you drink into glasses using the examples given below:

For spirits, liqueurs, and mixed drinks containing spirits, please count each nip (30 ml) as one glass.

1 can or stubby of beer = 2 glasses

1 bottle wine (750 ml) = 6 glasses

1 large bottle beer (750 ml) = 4 glasses

1 bottle of port or sherry (750 ml) = 12 glasses

17. Over the last 12 months, on days when you were drinking, how many glasses of beer, wine and/or spirits altogether did you *usually* drink?

TOTAL NUMBER OF GLASSES PER DAY	1	2	3	4	5	6	7	8	9	10 or more

18. Over the last 12 months, what was the *maximum* number of glasses of beer, wine and/or spirits that you drank in 24 hours?

MAXIMUM NUMBER OF GLASSES PER 24 HOURS	1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19 or more

FAT-Q

Below is a list of statements that describe how people sometimes feel. Please read each item carefully, then fill in the circle next to each item which best describes **how true each statement has been for you in the past 7 days.**

	Not at all	A little	Moderately	Quite a bit	Extremely
I have trouble remembering things	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My muscles ache	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel upset	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My legs feel weak	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel cheerful	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My head feels heavy	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel lively	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel nervous	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel relaxed	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel pooped	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am confused	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am worn out	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel sad	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel fatigued	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I have trouble paying attention	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My arms feel weak	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel sluggish	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel run down	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I ache all over	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am unable to concentrate	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel depressed	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel refreshed	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel tense	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel energetic	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I make more mistakes than usual	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
My body feels heavy all over	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am forgetful	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel tired	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I feel calm	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
I am distressed	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

GENERAL HEALTH AND INFORMATION - GHI

Today's Date / /

Your Service Number

Your Age years

Do you have children? ☐ Yes
☐ No

If yes, how many children?

YOUR HEALTH

Have you had vitamin/mineral supplements in the last 2 weeks? ☐ Yes
☐ No

Have you taken any antibiotics in the last 2 months? ☐ Yes
☐ No

Do you regularly (most days) take medication, vitamin supplements, or herbal preparations? ☐ Yes
☐ No

Product Name	Brand	Dosage (mg)	Doses taken per day
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>
<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>

Have you had a significant illness in the last 2 months? ☐ Yes
☐ No

Have you had major surgery in the last 2 months that required a general anaesthetic? ☐ Yes
☐ No

Do you smoke? ☐ Yes
☐ No

Have you had more than 2 average drinks in one session in the last week? ☐ Yes
(average drink=1 pot of beer, 1 glass wine, 1 nip/shot of spirits) ☐ No

Do you donate blood? ☐ Yes
☐ No

If yes, when did you last donate blood?

Are your periods regular (ie, spaced at regular intervals) ☐ Yes
☐ No

If yes, approximately how long is your cycle (eg 28 days)? days

Approximately how many days since the beginning of your last period? days

Approximately how many days bleeding do you have each period? days

Would you classify the level of bleeding ☐ Light
☐ Medium
☐ Heavy

Do you use ☐ The pill
☐ The injection
☐ Neither

Supplements

Tick off each day as you take your supplement:	M	T	W	T	F	S	S

Illnesses

Tick if you have experienced any of these illnesses/injuries this week:

Description	Code	✓	Description	Code	✓
Hayfever, cold, sore throat	URTI		Rashes, sores	FI	
Vomiting, upset stomach	GIT		Headache	HD	
Cuts, bleeding, bruising	TRA		Allergic reaction	AR	
Injuries to muscles or joints, sprains or strains	MUS		Flu, (fever, severe cold symptoms)	FLU	
Other (describe):					

Time spent in Classes

(you may attach a timetable instead of filling this out)

Day	Hours	Minutes
Monday		
Tuesday		
Wednesday		
Thursday		
Friday		
Weekend		

Physical Training on Duty

Please give details of your structured, timetabled PT, describing the activity type, for this week:

Day	Activity Description	Hours	Minutes
Monday			
Tuesday			
Wednesday			
Thursday			
Friday			
Weekend			

Appendix C: Analysis of overall dietary intake

Distribution of nutrient intakes below the Recommended Military Dietary Intake (RMDI) cut-offs were determined and combined with probability statistics to calculate the number of participants likely to have reported intakes below their individual requirements. This approach recognises that the RMDI overestimates nutrient requirements of almost all individuals in the population [1, 2].

There was no change in mean nutrient consumption from dietary sources by officer and staff cadets (paired t tests, $p > 0.05$) and there was no difference in either dietary intake or eating patterns between the treatment and placebo groups. Officer and staff cadets' weights remained stable at a mean of 65 kg (range 51.0–82.5 kg) throughout the semester. A high proportion of participants were at risk of not eating sufficient of one or more nutrients to meet their requirements [Table 1, Figure 1]. About one-third of the participants were at risk of eating insufficient dietary iron to meet their nutritional requirements (RMDI) in the year preceding the study. The nutrients most at risk for this group were carbohydrate and vitamin E.

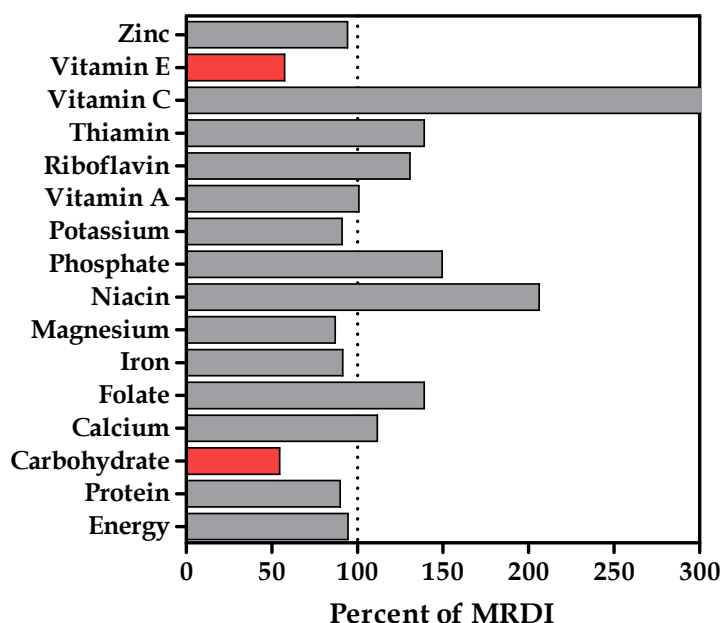


Figure 1. Nutrient intake by officer and staff cadets during the study semester expressed as a percentage of RMDIs. Nutrients highlighted in red represent mean daily intakes less than 75% of the MRDI.

Food risk was calculated by comparing each participant's intake of the major food groups against the "Core Food Groups" of the National Health and Medical Research Council (NHMRC) [Table 1] [3]. Based on their average number of food serves in each core food group, recruits were rated either "0" for meeting the core food group recommended serves or "1" for being outside the recommendation (e.g. fruit + vegetables + dairy products + meat + cereal products + extras gives a score from 0 = excellent to 6 = poor). Figure 2 presents the risk scores.

Table 1: Core Food Groups & Suggested Serves per Day

	Approx Energy per serve (kJ)	Example Serves	Serves (female)
Cereal	600	2 slices bread, 1 med bread roll 1 cup cooked rice, pasta or noodles 1 cup cooked porridge, 1.3 cup RTE cereal ½ cup untoasted muesli 1/3 cup flour	5
Vegetable	75 - 250	½ cup cooked vegetables ½ cup cooked dried beans, peas or lentils 1 cup salad vegetables 1 small potato	5
Fruit	300	1 medium piece of fruit 2 small pieces of fruit 1 cup diced fruit pieces or canned fruit 1 ½ tablespoons sultanas, dried fruits ½ cup fruit juice	2
Dairy	375-730	1 cup milk (fluid) 1 cup soy milk 1.2 cup evaporated milk 2 slices cheese 1 small carton yoghurt	3
Meat	600-850	65-100 g cooked meat or chicken ½ cup cooked beans, lentils, chickpeas, split peas or canned beans 80-120 g cooked fish fillet 2 small eggs 1/3 cup peanuts, almonds ¼ cup sunflower seeds, sesame seeds	1
Extra	600	1 doughnut 4 plain sweet biscuits 1 slice cake (40 g) 25 g chocolate (1/2 small bar) 2 tablespoons cream, mayonnaise 1 tablespoon butter, margarine, oil 1 ½ tablespoon sugar 200 mL wine 60 mL spirits 600 mL light beer 400 mL regular beer 1 can soft drink 1 small packet potato crisps (30 g) 1/3 meat pie or pasty 12 hot chips 1 ½ scoops icecream	2

Table 2 presents mean nutrient intakes as compared with the RMDIs and gives an estimate for the risk of dietary inadequacy for the study group. Because the intakes were similar, results for the 2003 semester only are included in the table.

Table 2: Mean daily (dietary) intake and estimate of inadequacy of nutrients as compared with the MRDIs

Nutrient	Mean \pm SD ^a	Range	RMDI	Percentage of group at risk of inadequate intake
Energy (MJ) ^a	8.6 \pm 2.4	4.7–15.1	9.1	60.5%
Protein (g)	90.0 \pm 27.4	43.3–177.2	100	34.8%
Carbohydrate (g)	217.9 \pm 57.6	111.8–313.9	400	85.6%
Fibre (g) ^b	18.8 \pm 6.1	10.6–31.5	(30)	-
Alcohol (g) ^c	15.3 \pm 15.6	0.4–49.2	-	-
Vitamin C (mg)	149.3 \pm 62.1	50.4–323.9	40	0.0%
Thiamin (mg)	1.7 \pm 0.5	1.0–3.4	1.2	3.5%
Riboflavin (mg)	2.4 \pm 0.8	1.3–5.4	1.8	6.3%
Folate (μ g)	278.0 \pm 73.8	162.4–442.6	200	2.7%
Niacin (mg)	39.2 \pm 11.9	19.9–79.1	19	0.0%
Vitamin A (μ g)	756.7 \pm 245.3	342.5–1186.2	750	28.4%
Vitamin E (mg)	5.8 \pm 1.8	2.6–9.8	10	82.9%
Magnesium (mg)	278.7 \pm 76.4	159.0–421.2	320	35.8%
Calcium (mg)	894.1 \pm 247.7	503.7–1483.2	800	20.3%
Phosphorus (mg)	1495.8 \pm 426.4	803.4–2457.2	1000	2.4%
Zinc (mg)	11.32 \pm 3.82	5.0–23.4	12	31.8%
Iron (mg)	12.8 \pm 3.7	6.3–19.3	13	34.2%

^a $n = 38$

^b Energy requirements were estimated as being an activity factor of 1.5, other requirements were based on Forbes-Ewan C. "ADF Nutrient Requirements in the 21st Century – A Report on the Nutrient Requirements of ADF Members Engaged in Base, Operational and Training Activities" Defence Nutrition Research Centre, February 2002.

^c The National Health and Medical Research Council (NH&MRC) gives as a guideline a recommended minimum dietary intake of 30 g fibre per day.

^d The NH&MRC gives as a guideline a maximum daily intake of 40g for men and 20g for women.

The only discernable difference in eating pattern was that the mean daily consumption of vegetable serves declined during 2003 (Table 3, $t = 2.063$, $p = 0.042$). However, this was not a sufficient change to alter the mean dietary risk score ($t = 0.135$, $p = 0.893$, Figure 2).

The Australian dietary guidelines are that total dietary fat intake should contribute no more than 28% of dietary energy with no more than 10% being from saturated fatty acids and at least 14% being from monounsaturated fatty acids and 6% from polyunsaturated fatty acids. The cadets' mean reported daily energy intake was

Table 3: Mean daily serves of the major food groups eaten by officer and staff cadets ^a

Food Group	2002		Semester 1 2003		Recommended for females
	Mean \pm SD	Range	Mean \pm SD	Range	
Bread, cereals, rice, pasta, noodles	4.3 \pm 2.0	1.0 – 9.0	4.6 \pm 2.0	0.8 – 9.0	5
Vegetables, legumes*	3.4 \pm 2.7	0.0 – 11.8	2.8 \pm 1.7	0.5 – 6.8	5
Fruit	3.1 \pm 1.8	0.0 – 9.5	3.0 \pm 1.8	0.0 – 7.0	2
Milk, yoghurt, cheese	2.2 \pm 1.0	0.5 – 5.5	2.1 \pm 0.8	0.5 – 3.6	3
Meat, fish, poultry, eggs, nuts, legumes	1.7 \pm 1.1	0.0 – 5.3	1.7 \pm 1.1	0.0 – 5.3	1
^b Extra foods and alcohol	3.6 \pm 2.3	0.3 – 13.2	3.9 \pm 2.2	0.3 – 9.0	2

^a Significant differences between food group intakes at $p < 0.05$ (*) are indicated. ^b Alcoholic beverage was a major contributor to this group.

8.6 MJ, with 33.6% of energy derived from total fats, 14.4% from saturated fats, 11.6% from monounsaturated fats and 4.7% from polyunsaturated fats. Figure 2 shows that most staff and officer cadets had a diet score of *average to poor*.

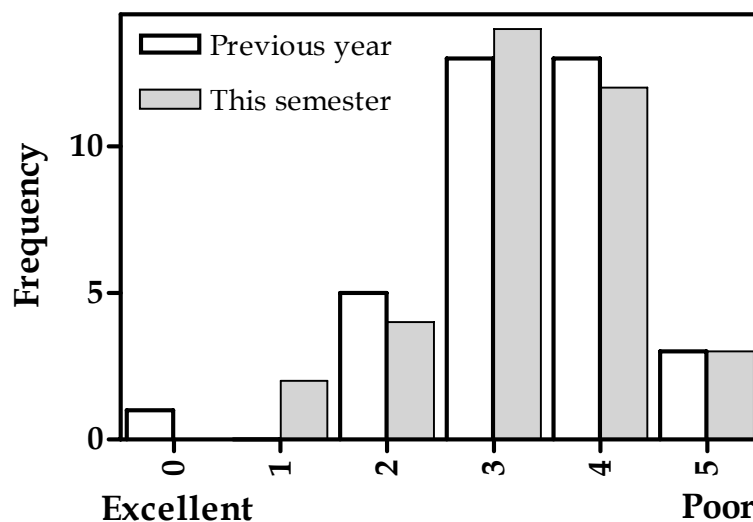


Figure 2. Risk scores based on consumption of core food groups for female officer and staff cadets.

1. Forbes-Ewan, C. H. (2002) *ADF nutrient requirements in the 21st century*, TA00235, DSTO Platform Sciences Laboratory

2. Anderson, G., Peterson, R. and Beaton, G. (1982) Estimating nutrient deficiencies in a population from dietary records: the use of probability analyses, *Nutr Res*, **2**, 409-415.
3. Department of Health and Family Services (1998) *The Australian guide to healthy eating: background information for nutritional educators*, DHFS, Canberra, Australia.

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Low Dose Ferrous Gluconate Supplement Fails to Alter the Iron Status of Female Officers-in-Training

Julia Carins, Christine Booth, Ross Coad

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19. ABSTRACT Physical training creates an iron cost for the body, which is a risk for young women. This study investigated a low-dose iron supplement for prevention or treatment of iron-deficiency among female RMC staff cadets and ADFA officer cadets and in so doing improve measures of fatigue, general health, physical fitness and increase participation in leisure activities. Cadets consumed either a low dose iron supplement (18 mg iron) or placebo for 13 weeks, using a double-blind, placebo-controlled randomised design. Tests at baseline, 6 wks and 13 wks determined the effects of supplement versus placebo on iron status and other measures. There was no evidence of benefit derived from the iron supplement, although emotional fatigue might have responded positively. The fatigue, health and leisure activity measures remained stable. Physical fitness improved at 6wks, but the improvement had been lost by 13 wks. Early in the semester, when cadets were most physically active, there was a mean decline in iron status as iron was mobilised from liver stores to the tissues. By the end of the semester the apparent loss from iron stores had been replenished. However more than half of the young women commenced the study with iron deficiency to some degree and this situation did not change at the 6 wk or 13 wk testing points. Self-administration of iron supplements is not recommended for the prevention or treatment of iron deficiency. The implementation of nutrition and iron-status monitoring programs are recommended.					